Smad7 Sensitizes Tumor Necrosis Factor–Induced Apoptosis through the Inhibition of Antiapoptotic Gene Expression by Suppressing Activation of the Nuclear Factor-κB Pathway

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Abstract
Although tumor necrosis factor (TNF) induces apoptosis and cell death in many tumor cells, some cancer cells are still resistant to the TNF-induced death signal. In this report, we showed that Smad7, an inhibitory Smad of transforming growth factor-β (TGF-β) signaling, can overcome the TNF resistance in human breast and gastric cancer cells. Overexpression of Smad7 induces the degradation of poly(ADP-ribose) polymerase and the activation of caspase cascade. Although c-jun NH2-terminal kinase (JNK) signaling is involved in TNF-induced cell death, the expression of Smad7 does not synergize the activation of JNK. However, the activation of nuclear factor-κB (NF-κB), the cell survival factor, is markedly decreased in Smad7-stable cells. Furthermore, the expression of antiapoptotic target genes of NF-κB is significantly reduced in accordance with the level of Smad7. In addition, Smad7 mediates the inhibitory activity of TGF-β on TNF-induced NF-κB activation and the synergistic activity of TGF-β on TNF-induced apoptosis. These findings suggest that Smad7 sensitizes the tumor cells to TNF-induced apoptosis through the inhibition of expression of antiapoptotic NF-κB target genes. [Cancer Res 2007;67(19):9577–83]

Introduction
Response to various environmental stimuli, including cell death or proliferation signal, results in different outcomes dependent on cell contexts. Tumor necrosis factor (TNF) has been known as a major mediator of apoptosis as well as inflammation and tumorigenesis and has been implicated in the pathogenesis of a wide spectrum of human diseases (1). After interacting with its receptors, TNF induces apoptosis through the recruitment of TNF receptor 1 (TNFR1)-associated death domain protein and Fas-associated death domain. These complexes stimulate the extrinsic pathway through the cleavage and activation of caspases (1, 2). In addition to apoptotic signals, TNF also induces cell survival signals by recruiting the intracellular adaptor molecules receptor-interacting protein (RIP), TNF receptor-associated factor (TRAF) 1, and TRAF2 (3). TRAF2 plays a critical role in activation of downstream proteins mediated by both TNFR1 and TNFR2 and has been known in TNF-induced activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPK; ref. 4). The NF-κB is a family of stimulus-induced homodimeric or heterodimeric transcription factors that plays important roles in developmental and immune processes (5). In resting cell, NF-κB is localized in cytoplasm by interacting with inhibitory proteins (IκBs). When cells are stimulated by exogenous stimulus, IκBs are phosphorylated by upstream IκB kinase. This triggers the ubiquitination and degradation of IκB by proteasome pathway. The free NF-κB can be translocated into the nucleus and activate the transcription of target genes. NF-κB pathway also has a role in tumor initiation, survival, and progression (6–8).

The transforming growth factor-β (TGF-β) superfamily consists of a large number of structurally related polypeptide growth factors, such as TGF-β, bone morphogenetic protein (BMP), and activins, which play critical roles in cell proliferation, differentiation, motility, adhesion, and death (9). TGF-β sends the signals through the interaction with heteromeric complexes of type II and type I serine/threonine kinase receptors (TβRII and TβRI) and their interaction partners, the Smads proteins (10). The binding of the TGF-β ligand to type II receptor facilitates the initiation of signaling through the phosphorylation of type I receptor, which subsequently phosphorylates the receptor-regulated Smads, such as Smad2 and Smad3. Activated Smad2 and Smad3 are leading to binding with the common mediator Smad, Smad4, and these protein complexes are translocated from the cytoplasm to the nucleus. These Smad complexes can act as coactivators or corepressors in the nucleus through the interaction with another transcription factors.

Smad7 is an inhibitory Smad, which functions to block TGF-β–induced Smad activation and to terminate the signaling pathway as feedback regulation (11). Smad7 localizes in the nucleus by interacting with E3 ubiquitin ligases Smurfl or Smurfr2 in resting state and the Smad7–Smurf complex exports to the cytoplasm on TGF-β treatment. In the cytoplasm, Smurf interacts with TGF-β receptors and then ubiquinatates and degrades the receptors via proteosomal pathway. In addition, Smad7 can inhibit the TGF-β signaling through interfering the interactions between the Smad proteins and the activated receptors.

Although Smad7 was originally identified as an inhibitory Smad of TGF-β, there are several reports that suggest that inhibitory Smads may have another cellular functions and can cooperate with TGF-β to show some activities. Smad7 is involved in TGF-β–induced apoptosis of prostate cancer cells by activating p38 via TGF-β–activated kinase 1 (TAK1) and MAPK kinase (MKK) 3 (12). In another report, overexpression of Smad7 by adenoviral infection induced DNA fragmentation and significant increases in cell death ELISA and caspase-3 assay in rat mesangial cells (13). In contrast, another Smads, Smad2 or Smad3, had not shown any significant increases in caspase-3 activity. Most importantly, TGF-β–induced

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apoptosis was prevented by inhibition of Smad7 expression, by antisense mRNA in stably transfected cell lines, or on transient transfection with antisense oligonucleotides. Recently, we have shown that Smad7 inhibits TNF-induced NF-κB activity by blocking TAK1-associating proteins, TAB2 and TAB3, from forming a complex with TRAF2 (14). Therefore, in this report, we examined whether inhibition of TNF-induced NF-κB activity by Smad7 sensitizes TNF-induced apoptosis. We show that Smad7 sensitizes the human tumor cells to TNF-induced apoptosis through the inhibition of the survival signal NF-κB activation.

Materials and Methods

Cell culture and reagents. SNU638 human gastric cancer cells established from gastric carcinomas of patients and MCF7 human breast cancer cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37°C in a 5% CO₂ atmosphere. Stably Smad7-expressing cells were generated by retroviral infection of LPCX-Flag-Smad7 as described (15) and maintained with 400 ng/mL puromycin. HeLa cell was grown in DMEM with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin in a 37°C incubator with 5% CO₂. Caspase-8 p20 (H134), Smad7 (N19 or P20), IκBα (FL), and c-Jun NH₂-terminal kinase (JNK) 1 (FL) antibodies were purchased from Santa Cruz Biotechnology. The phosphorylated MAPK and caspase-3 antibodies were from Cell Signaling and Flag (M2) and h-actin (clone AC-15) were obtained from Sigma. Anti-poly(ADP-ribose) polymerase (PARP) antibody was purchased from Upstate.

Transient transfection and luciferase assay. Transfection and reporter assay were done as described previously (15). Briefly, MCF7 cells were seeded in 12-well plates at 1 × 10⁵ per well as triplicates and transiently transfected with NF-κB reporter and Smad7 expression plasmid. A β-galactosidase DNA was used as an internal control. We used LipofectAMINE Plus as transfection agents according to the manufacturer's instructions (Invitrogen). After 6 h, medium was changed with serum-containing medium and treated with TGF-β1 or TNF following 12 h. All assays were done in triplicate, and data shown represent the mean of three independent experiments.

Design and transfection of Smad7 small interfering RNA. Specific small interfering RNA (siRNA) for Smad7 was made from Dharmacon, Inc. Two human Smad7-specific sequences were used, siRNA1 (5’-GAAACT-GAAGGAGCGGCAG-3’) and siRNA2 (5’-ATTCGGACAACAAGAGTCA-3’), according to nucleotides 303 to 321 and 905 to 923, respectively (Genbank accession number AF015261). siGENOME SMARTpool SMAD7 siRNA was purchased from Dharmacon. MCF7 and HeLa cells were seeded at 60% density in six-well plate. After 24 h, transfection was done using LipofectAMINE 2000 according to the manufacturer's instructions (Invitrogen).

Western blotting. The cells were treated with TNF (10 ng/mL) or TGF-β1 (5 ng/mL) for indicated times and cells were lysed in a buffer containing 25 mmol/L HEPES (pH 7.5), 150 mmol/mL NaCl, 1% Triton X-100, 10% glycerol, 5 mmol/mL EDTA, and protease inhibitor cocktails (Roche). For immunoblotting, cell debris was removed by centrifugation and proteins were separated by SDS-PAGE followed by transfer to polyvinylidene difluoride membrane. After incubating with the appropriate primary antibodies, proteins were visualized by chemiluminescence according to the manufacturer's instructions (Pierce).

Reverse transcription-PCR and Northern blot. Trizol reagent (Invitrogen) was used for the isolation of total RNA from MCF7-LPCX or Smad7 cells, which were treated with TNF for 24 h. For reverse transcription-PCR (RT-PCR), we used OneStep RT-PCR kit (Qiagen) according to the manufacturer's instructions. Samples were normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels.

Figure 1. Smad7 sensitizes the cells to apoptosis. A. MCF7 or SNU638 cells stably expressing Smad7 were treated with TNF (10 ng/mL) for 24 h. The morphologic changes were photographed. B, MCF7-LPCX or Smad7 cells were treated with TNF (10 ng/mL) for 24 h, and cell lysates were immunoblotted with anti-PARP and anti-Flag antibody. The β-actin antibody was used as internal control.
Smad7 siRNA reduced caspase-8 and PARP cleavage induced by Smad7. Knockdown of Smad7 expression by transfection with Smad7-specific siRNA in MCF7 cells stably expressing caspase-8 activation. To confirm this finding, we examined that the apoptosis induced by Smad7 and TNF is mediated via the caspase cascade in apoptosis mediated by Smad7 and TNF, we tested the effect of a specific caspase-8 inhibitor (z-IETD-fmk) as well as a general caspase inhibitor (z-VAD-fmk) on cell death induced by TNF treatment in Smad7-expressing MCF7 cells. Both inhibitors effectively blocked PARP cleavage in cells treated with TNF, concomitant with caspase-8 and caspase-3 inhibitions (Fig. 2B). Similar results were also found in Smad7-expressing SNU638 cells treated with TNF (data not shown). These results thus indicate that the apoptosis induced by Smad7 and TNF is mediated via caspase-8 activation. To confirm this finding, we examined the effect of Smad7-specific siRNA in MCF7 cells stably expressing Smad7. Knockdown of Smad7 expression by transfection with Smad7 siRNA reduced caspase-8 and PARP cleavage induced by the TNF treatment (Fig. 2C). These results suggest that Smad7 enhances the apoptotic activity of TNF in MCF7 and SNU638 cells.

Smad7 does not activate JNK. Previous reports showed that the JNK pathway plays an important role in TNF-induced apoptosis (18). To see the involvement of the MAPK pathway in Smad7 sensitization of TNF-induced apoptosis, we used MAPK inhibitors in MCF7 cells expressing Smad7. PARP cleavage was decreased by treatment with JNK inhibitor SP600125, but SB203580, an inhibitor of p38, and U0126, an inhibitor of MAPK/extracellular signal-regulated kinase (ERK) kinase 1 (MEK1), did not show any inhibitory effects (Fig. 3). This finding also confirmed the involvement of the JNK pathway in TNF-induced apoptosis in MCF7 cells. We next examined the activation of MAPKs p38, JNK, and ERK by

Results

Smad7 sensitizes the cells to TNF-induced apoptosis. To understand the role of Smad7 in TNF-mediated apoptosis, we examined TNF-induced cell death in Smad7-stable cell lines. The majority of cancer cells are resistant to TNF-induced apoptosis. MCF7 human breast cancer cells and SUN638 human gastric cancer cells were also resistant to TNF-induced cytotoxicity as shown by the cellular morphology (Fig. 1A). However, TNF markedly induced cell death in MCF7 and SNU638 cells stably expressing Smad7 (Fig. 1A). No evident cell death was observed when these cells were cultured in the absence of TNF, suggesting that Smad7 expression greatly sensitizes MCF7 and SNU638 cells to TNF-induced cell death. When cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, TNF treatment markedly enhanced caspase-8 cleavage in MCF7 and SNU638 cells expressing Smad7 (Fig. 2). Caspase activation is required for apoptosis. Smad7 sensitizes TNF-induced apoptosis via the caspase receptor pathway initiating from TNF treatment (Fig. 2C). These results suggest that Smad7 enhances the apoptotic activity of TNF in MCF7 and SNU638 cells.

![Figure 2. Caspase activation is required for apoptosis. A, SNU638 cells expressing Smad7 were treated with TNF for 24 h, and cell lysates were immunoblotted with anti-caspase-3, anti-caspase-8, or anti-PARP antibody. A nonspecific band (*) was detected by the caspase-8 antibody. B, MCF7 cells stably expressing Smad7 were treated with TNF in the presence or absence of 10 μmol/L of z-IETD-fmk or z-VAD-fmk for 24 h. The cell lysates were immunoblotted with anti-PARP antibody or caspase-8 antibody. C, MCF7 cells stably expressing Smad7 were transfected with 100 nmol/L siRNAs against Smad7 or scramble (SCR) followed by treatment with TNF for 24 h. The lysates were detected with anti-caspase-8 or anti-PARP antibody.

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with the following primers: GAPDH, 5′-GTCGGAGTCAACGGATT-3′ (forward) and 5′-AACGTGCCGTGTCCAG-3′ (reverse); cellular inhibitor of apoptosis protein-1 (cIAP-1), 5′-TCAACTCTCATCAATACTCATT-3′ (forward) and 5′-GCTTTGACATCATCTGTTACCC-3′ (reverse); FLICE inhibitory protein (FLIP), 5′-TCCAGAAGAAGCAGCTGTTC-3′ (forward) and 5′-GAAGTGTGCTGTCGCCACACA-3′ (reverse); and X-linked inhibitor of apoptosis (XIAP), 5′-TTTCCATTAGTGAATCTAGCTGTT-3′ (forward) and 5′-GTACCAGTTGTTTGGAAATG-3′ (reverse). For Northern blot analysis, 15 μg RNA was electrophoresed on 1% agarose gels and transferred to a nitrocellulose membrane. Blots were hybridized with β-D-labeled cDNA probes for cIAP-1, FLIP, XIAP, and GAPDH. After overnight incubation, membranes were washed thrice and then visualized by autoradiography.

Results

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TNF in MCF7 cells stably expressing Smad7. However, the activity of JNK was not enhanced in MCF7-Smad7 cells (Fig. 3B), suggesting that Smad7 does not induce apoptosis through the enhancement of JNK activity. TNF-induced activation of p38 was markedly decreased in MCF7-Smad7 cells; however, the significance of this decrease is not clear because p38 is not involved in TNF-induced apoptosis in MCF7-Smad7 cells. To eliminate the possibility of involvement of reduced activation of p38 in apoptosis by Smad7, we used the p38 wild-type or constitutive active MKK6 constructs. Transient expression of these constructs induced the constitutive activation of p38 in MCF7-Smad7 cells (Supplementary Fig. S2). Although p38 is highly activated in transfected MCF7-Smad7 cells, TNF still induced apoptosis compared with mock-transfected cells, suggesting that a lower level of p38 activation by TNF in MCF7-Smad7 cells does not contribute to higher apoptosis (Supplementary Fig. S3). The basal activity of ERK was high in control MCF7 cells, but TNF-induced activation was delayed in MCF7-Smad7 cells.

**TGF-β1 inhibits TNF-induced NF-κB activation through the induction of Smad7.** We next examined whether TGF-β1 inhibits TNF-induced NF-κB activation through the induction of Smad7. First, we checked whether TGF-β1 blocks TNF-induced IκBα degradation. For this purpose, we used HeLa cells because both MCF7 and SNU638 cells do not respond to TGF-β1 due to the lack of TGF-β type II receptor expression (19, 20). As shown in Fig. 4A, treatment of TNF significantly increased IκBα degradation, and TGF-β1 pretreatment for 24 h almost completely blocked TNF-induced IκBα degradation in HeLa cells. We observed slow induction of Smad7 in HeLa cells after TGF-β1 treatment. The level of Smad7 induced by TGF-β1 correlated with the inhibition of IκBα degradation. Shorter TGF-β1 pretreatment did not block the degradation of IκBα, suggesting that certain levels of Smad7 protein are required to inhibit TNF-induced IκBα degradation. We also examined whether TGF-β1 inhibits TNF-induced NF-κB transcriptional activity, and results showed that TGF-β1 pretreatment also inhibited TNF-induced NF-κB transcriptional activity (Supplementary Fig. S4). To see the effect of TGF-β1 on TNF-induced caspase-3 activation as an apoptotic marker, HeLa cells were pretreated with TGF-β1 for the indicated times followed by treatment with TNF for another 24 h. Treatment of TNF or TGF-β1 alone did not induce caspase-3 activation significantly, but pretreatment of TGF-β1 markedly increased the apoptotic activity of TNF (Fig. 4B). Expression of Smad7 was increased in accordance with treatment of TGF-β1. Increased levels of Smad7 and enhanced Smad2 phosphorylation were maintained even 48 h after TGF-β1 treatment (Supplementary Fig. S5). To see whether Smad7 induced by TGF-β1 mediates inhibition of TNF-induced NF-κB transcriptional activity and apoptosis by TGF-β1, we used Smad7 siRNA to reduce expression of endogenous Smad7 protein. The SMARTpool Smad7 siRNA was used to knock down the endogenous expression of human Smad7. HeLa cells were transfected with 100 nmol/L of Smad7 siRNA or scrambled siRNA. Low levels of Smad7 were expressed in HeLa cells in the absence of TGF-β1. TGF-β1 treatment induced expression of Smad7 in HeLa cells transfected with the control scrambled siRNA, whereas Smad7 protein levels decreased by 80% when Smad7 siRNA was transfected. Knockdown of Smad7 expression by the Smad7 siRNA transfection resulted in an increase in TNF-induced IκBα degradation even in the presence of TGF-β1 (Fig. 4C). In accordance with the inhibitory activity of Smad7 on TNF-induced NF-κB activation, knockdown of Smad7 blocked the synergistic activity of TGF-β1 on TNF-induced caspase-3 activation as well as PARP degradation (Fig. 4D). This result suggests that TGF-β1 can enhance TNF-induced apoptosis through the inhibition of NF-κB activation.

**Smad7 suppresses the expression of NF-κB antiapoptotic target genes.** The antiapoptotic function of NF-κB is dependent on the expression of various target genes, such as XIAP, FLIP, and cIAP-1. Here, we further examined whether Smad7 influences the...
expression levels of those genes. Cells were treated with TNF for 24 h, the total RNA was prepared, and RT-PCR and Northern blot were done (Fig. 5A and B). As shown in Fig. 5, TNF markedly up-regulated the expression of XIAP, FLIP, and cIAP-1, whereas Smad7 expression significantly reduced their expression levels. XIAP, FLIP, and cIAP-1 are important antiapoptotic molecules (21), and their reduced expression levels caused by Smad7 expression are likely to contribute to the sensitization effect of Smad7 on TNF-induced apoptosis.

Figure 4. TGF-β1 inhibits TNF-induced IκBα degradation. A, HeLa cells were pretreated with TGF-β1 for 3, 8, or 24 h followed by treatment with TNF for 20 and 60 min. Cell lysates were prepared and Western blot was done using anti-IκBα, anti-phosphorylated Smad2 (p-Smad2), and anti-Smad7 antibodies. The relative intensity of IκBα was determined using NIH ImageQuant program. B, HeLa cells were treated with TGF-β1 for 16 or 24 h followed by treatment with TNF for another 24 h. Expression of caspase-3 as an apoptotic marker was detected with Western blotting and normalized with β-actin. C, HeLa cells were transfected with scramble (si-Scr) or siRNAs against Smad7 and treated with TGF-β1 for 24 h followed by treatment with TNF for 30 min. The level of IκBα and Smad7 was detected with anti-IκBα and anti-Smad7 antibody. D, HeLa cells were transfected with scramble or Smad7-specific siRNA for 48 h and then pretreated with TGF-β1 for 24 h. Apoptotic activity of TNF was detected by Western blotting against caspase-3, PARP, and procaspase-8 after additional treatment with TNF for 24 h. Smad7 levels were detected with Smad7-specific antibody. *, the procaspase-8 antibody also detected the nonspecific band.

Figure 5. Smad7 inhibits the expression of antiapoptotic genes. A, total RNA was extracted from the MCF7 cells after treating TNF for 24 h and RT-PCR was done using specific primers for cIAP-1, FLIP, and XIAP. GAPDH was used for internal control. The same RNA was used for Northern blotting to confirm the result of RT-PCR. B, 15 μg total RNA was loaded on each well and GAPDH was used as loading control. C, schematic diagram of cross-talk between TGF-β1 and TNF signaling. Smad7, induced by TGF-β1, can sensitize the cells to TNF-induced cell death through the blocking of the activation of NF-κB target genes. In contrast, Smad7 can be induced by TNF through the NF-κB pathway and inhibits the TGF-β1 signaling pathway.
Discussion

Resistance to death signal of tumor cell is the most important obstacles in treatment of many cancers (22, 23). It will be helpful to find out the molecular targets to understand the mechanism of resistance of tumor and to sensitize the tumor cell by treatment with reagents. Because activation of NF-κB plays a major role to protect cells against apoptotic stimuli, such as TNF and Fas ligand, it has been known as good therapeutic target for treatment of cancer (24). In this study, we have shown that Smad7 expression greatly sensitizes MCF7 and SNU638 cells to TNF-induced cell death, suggesting that inhibition of NF-κB-mediated antiapoptotic signals may tip the balance in favor of TNFR1-activated apoptotic signals. We have previously shown that overexpression of Smad7 substantially suppresses TNF-induced NF-κB activation (14). These findings are consistent with previous reports that induction of Smad7 inhibits transcriptional activity of NF-κB in Madin-Darby canine kidney cells and in conditionally immortalized mouse podocytes (25, 26). Transfection with Smad7-specific siRNA increased TNF-induced NF-κB transcriptional activity, showing that endogenous Smad7 acts as a negative modulator in TNF signaling.

Activation of the JNK pathway is critical for TNF-induced apoptosis (18). We have also shown that the JNK pathway is important for TNF-induced cell death in MCF7 cells (Fig. 3A). Although the activation of JNK is important for TNF-induced apoptosis, there were no significant differences in TNF-induced JNK activity between control and Smad7-expressing MCF7 cells, suggesting that Smad7 does not activate JNK (Fig. 3B). A recent report showed that the ubiquitination and translocation of TRAF2 to the insoluble fraction is critical for activation of JNK but not of p38 or NF-κB (27). However, Smad7 does not regulate the TNF-induced TRAF2 ubiquitination (14). In addition, RIP-deficient mice showed extensive apoptosis in both the lymphoid and adipose tissue and died at 1 to 3 days after birth. Rip1−/−MEF cells are highly sensitive to TNF-induced cell death, which is caused by a failure to activate NF-κB, but not JNK (28). We found that Smad7 has no effect on RIP1 activity (14). Apoptosis signal-regulating kinase 1 (ASK1) is involved in TNF-induced JNK activation through the interaction with TRAF2 (29). However, the effect of Smad7 on ASK1 activation is not known.

The relationship between TNF and TGF-β is important in apoptosis, inflammation, and cancer. In the TGF-β1−/−null mouse, the infiltration of mononuclear cells into vital organs is accompanied by the overexpression of IFN-γ and inducible nitric oxide synthase, and the resulting toxic levels of nitric oxide contribute to the lethal phenotype of the TGF-β1−/−null mice (30, 31). NF-κB activity is markedly increased in inflamed tissue and is thought to play a critical role in the pathogenesis of inflammatory and autoimmune diseases, such as inflammatory bowel disease (IBD; ref. 32) and rheumatoid arthritis (33, 34), making this factor a potential target for therapeutic intervention. In cell culture systems, TGF-β can either promote or inhibit NF-κB activation depending on the cell type used (35–38). TGF-β treatment of WEHI 231 and CH33 cells reduced nuclear Rel/NF-κB activity in part by an increase in the IκBα protein resulting from decreased gene transcription (35). TGF-β is also a negative regulator of NF-κB in human intestinal lamina propria mononuclear cells (39). Pretreatment of normal lamina propria mononuclear cells with TGF-β blocked TNF-induced NF-κB p65 accumulation in the nucleus, and this was associated with induction of IκBα. Recent study has shown that induction of IκBα by Smad7, thereby inhibiting NF-κB activation and NF-κB-driven inflammatory response, may be a key signaling pathway whereby TGF-β exerts its anti-inflammatory properties in vivo and in vitro (40). In addition, TGF-β and TNF can cooperate to induce apoptosis in several cell lines consistent with our result (41, 42).

Smad6 and Smad7 have been identified as inhibitors of the TGF-β, activins, and BMP signaling pathway, and inhibitory Smads function by interfering with the activation of receptor-regulated Smads through interaction with TGF-β type I receptor or other Smad proteins. Smad7 expression is rapidly induced by TGF-β family members in several cell types, indicating that Smad7 is in feedback regulation of TGF-β signaling. Recent studies suggest that Smad7 may serve as mediator of TGF-β in the cell rather than acting simply as an inhibitor of TGF-β signaling. In scleroderma, basal level and TGF-β–inducible expression of Smad7 are selectively decreased, and the introduction of Smad7 restores normal TGF-β signaling in scleroderma fibroblasts (43). In contrast, another study reported that scleroderma fibroblasts exhibited increased Smad7 levels compared with normal fibroblasts in vivo and in vitro (44). In T cells of IBD patients, increased Smad7 expression causes the blockade of TGF-β signaling and anti-inflammatory effects (45). Moreover, ectopic expression of Smad7 has been shown to induce apoptosis (25). In addition, expression of Smad7 in JygMC(A) cells decreased in metastasis to the lung and liver. This effect may be associated with increased expression of components of adherens and tight junctions, decreased expression of N-cadherin, and decreases in the migratory and invasive abilities of the JygMC(A) cells (46). These observations suggest that Smad7 is not simply an inhibitor of signaling pathways of TGF-β family members but may also function as a mediator of TGF-β signaling pathways. In this study, we have provided evidence that Smad7 may function as a scaffold protein of the cross-talk between different signaling pathways (Fig. 5C). Smad7, induced by TGF-β, an anti-inflammatory signal, blocks the activation of NF-κB, which is induced by proinflammatory cytokines, such as TNF or interleukin-1β (47). On the other hand, the TGF-β–signaling can be inhibited by Smad7, which is induced by the TNF and IFN-γ through NF-κB and signal transducers and activators of transcription pathways. Therefore, the balance between different signals and the proinflammatory and anti-inflammatory pathways seems to be important in cell fate for survival or cell death.

Because Smad7 sensitizes TNF-mediated apoptosis through the inhibition of TNF-mediated NF-κB activation, pharmacologic augmentation of Smad7 expression may be a useful therapeutic strategy in tumors that express high level of TNF. In previous report, halofuginone is known to induce expression of Smad7 dramatically and reduce the radiation-induced fibrosis (48). Interestingly, halofuginone has been shown to inhibit tumor growth through the blocking of angiogenesis and matrix metalloproteinase (49, 50). It will be interesting to screen the chemicals that induce the specific expression of Smad7 and examine the effect of chemicals whether it has proapoptotic activity through the induction of Smad7 in tumors.

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